Supporting Information

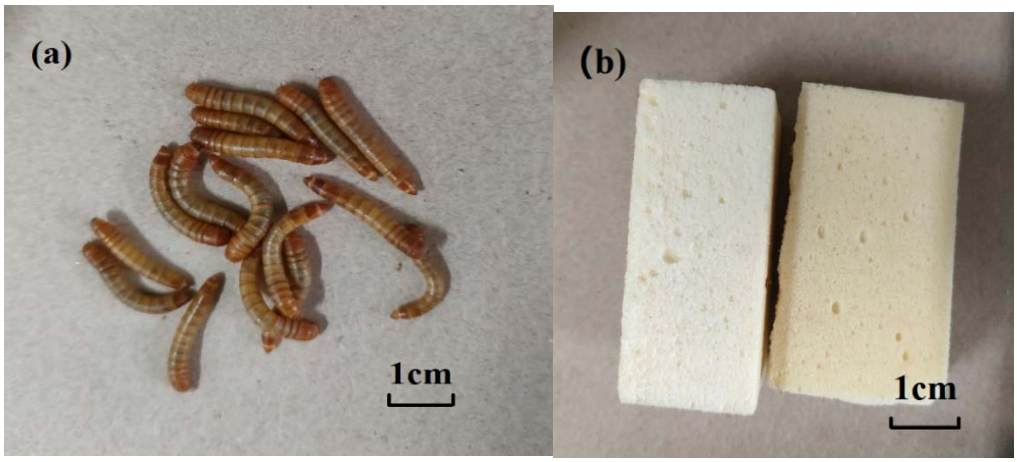

Fig. S1 Image of (a) mealworms; and (b) WRPU.

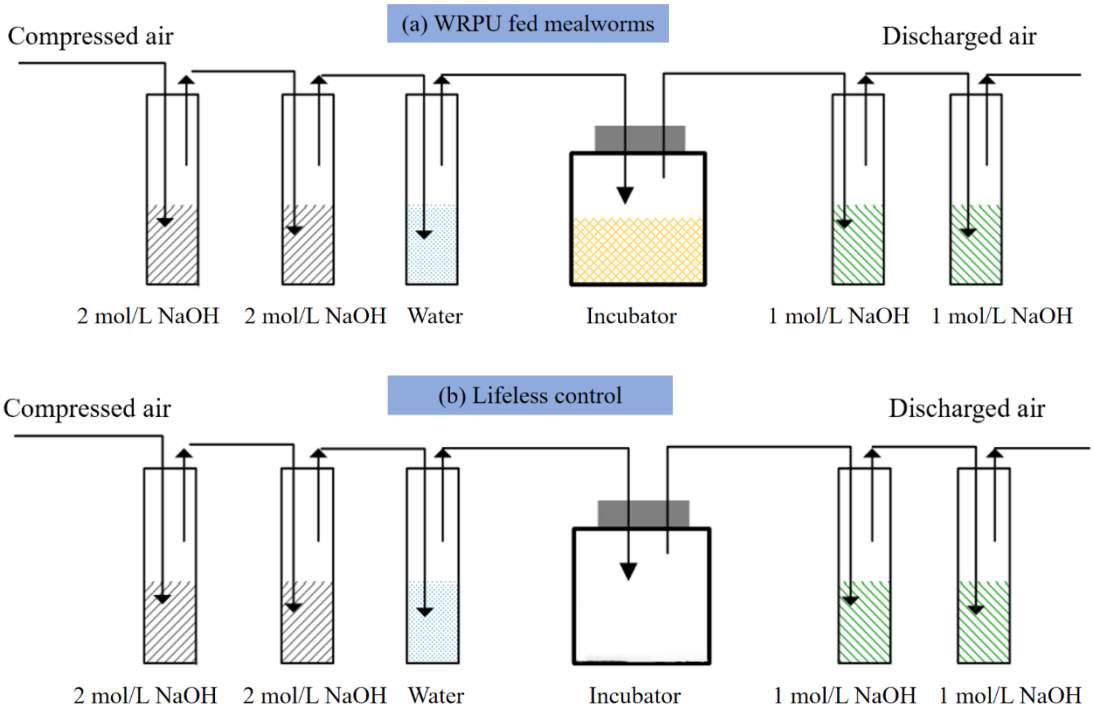

Fig. S2 The experimental set-up for determining carbon balance: (a) WRPU fed mealworms; (b) Lifeless control.

Methods

M1 Carbon balance analysis of the metabolism of mealworms fed with WRPU

For the analysis, the mealworms were fed with WRPU as the only diet in 12 incubators (1000 mL) sealed with rubber stopper, each containing 60 mealworms. A lifeless control was used which ensured that no CO_2 was generated (n=3). Compressed air was passed through two CO_2 trappers (2 mol/L NaOH, 500 mL) connected in series with glass tubes to remove CO_2 from the air, moisturize it, and then to enter the incubator. The off-air passed through another two CO_2 trappers (1 mol/L NaOH, 500 mL) in series with glass tubes to collect the CO_2 produced from the incubator (Fig. S2 in the Supporting Information). The CO_2 produced by each incubator was collected in 1 mol/L NaOH solution, after which $BaCl_2$ was added to produce $BaCO_3$ precipitation, dried to constant weight, and then weighed. The dry weight of $BaCO_3$ was used to calculate the CO_2 collected. The incubation time was 5, 10, 15, and 20 days, respectively. Pick out dead mealworms every day to avoid the live ones eating them. At the end of each incubation time, mealworms and the corresponding frass were dried to constant weight, after which the mass of ingested WRPU, the dry weight of mealworms, the total CO_2 generation, and the dry weight of corresponding frass were determined.

The calculation formula of ΔCarbon fraction of WRPU loss is

$$\begin{aligned}&\Delta\text{C of WRPU loss (mg)}\\&\quad=(\text{Initial WRPU mass}-\text{Final WRPU mass})\times \text{C}_{\text{WRPU}}\%\end{aligned}, \quad \text{(S1)}$$

C_{WRPU}%—— C% in WRPU;

The calculation formula of ΔCarbon fraction of frass residues is

$$\Delta\text{C of frass residues (mg)} = \text{Dry frass residues mass} \times \text{C}_{\text{frass}}\%, \quad \text{(S2)}$$

C_{frass}%——C% in frass residues;

The calculation formula for the conversion rate of carbon from WRPU loss to frass residues is

$$w\% = \left(\frac{\Delta\text{C of frass residues}}{\Delta\text{C of WRPU loss} + \Delta\text{C of biomass}}\right)\times 100\%, \quad \text{(S3)}$$

The calculation formula of ΔCarbon fraction of CO_2 release is

$$\Delta\text{C of CO}_2\text{ release (mg)} = \text{CO}_2\text{ mass of WRPU fed mealworms} \times 12/44, \quad \text{(S4)}$$

The calculation formula for the conversion rate of carbon from WRPU loss to CO_2 release is

$$w\% = \left(\frac{\Delta\text{C of CO}_2\text{ release}}{\Delta\text{C of WRPU loss} + \Delta\text{C of biomass}}\right)\times 100\%, \quad \text{(S5)}$$

The calculation formula of ΔCarbon fraction of biomass is

$$\begin{aligned}&\Delta\text{C of biomass (mg)} = (\text{Dry weight of dead mealworms} \times \text{C}_{\text{dead}}\% +\\&\text{at the end of the survival of mealworms dry weight} \times \text{C}_{\text{live}}\%) -\end{aligned}, \quad \text{(S6)}$$

$$\text{Initial mealworms mass} \times (1 - \text{Initial mealworms moisture content}) \times C_{\text{Initial}}\%$$

C_{dead}%——Carbon percentage in the dry weight of dead mealworms;

C_{live}%——Carbon percentage in the dry weight of live mealworms in the end;

$C_{Initial}$%—— Carbon percentage in the dry weight of initially added mealworms;

The calculation formula of the total C recovery of WRPU loss is

$$w\% = \left(\frac{\Delta\text{C of } CO_2 \text{ release} + \Delta\text{C of frass residues}}{\Delta\text{C of WRPU loss} + \Delta\text{C of biomass}}\right) \times 100\% \quad , \quad \text{(S7)}$$

M2 Gut microbial community method

The gut genomic DNA was extracted using the E.Z.N.A.® soil DNA kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's instructions, and all DNA samples were quality checked and the concentration was quantified by NanoDrop 2000 spectrophotometers (Thermo Fisher Scientific, Wilmington, DE, USA). Phased amplicon sequencing was used to sequence the V3–V4 region of the 16S rRNA gene. All samples were processed according to the formal experimental conditions, and each sample was repeated three times. The PCR products of the same sample was mixed and verified from a 2% agarose gel electrophoresis. Then the resulted PCR products were further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, AXYGEN, San Francisco, USA). Based on preliminary quantitative results of electrophoresis, the PCR products were quantified using QuantiFluor™ -ST Bule Fluorescence Quantification System (Quantus™ Fluorometer, Promega, Madison, USA) and then mixed according to the sequencing amount of each sample. Purified amplicons were paired-end sequenced on an Illumina MiSeq platform (Illumina, San Diego, USA) (Peng et al., 2019; Yang et al., 2021). Sequencing data was demultiplexed, quality filtered, and combined according to criteria to obtain optimized data (Zhang et al., 2014).

Sequencing reads were partitioned to sequences represented by amplicon sequence variant (ASV) using DADA2. The classification of each 16S rRNA gene sequence was analyzed by the Naive Bayes classifier against the Silva 16S rRNA database. Eventually, the free online platform of the Majorbio I-Sanger Cloud Platform (Shanghai, China) was used to run microbial community composition, alpha diversity analysis, and principal coordinate analysis (PCoA).

Table S1 Elemental composition of the WRPU and the corresponding frass (mean ± SD; *n*=3).

Element (wt.%)	WRPU	Frass
C	55.339±1.632	42.718±2.101
N	16.542±0.871	25.892±1.120
O	26.115±1.411	24.122±0.093
P	0.016±0.023	3.001±0.010
Cl	1.704±0.162	1.593±0.042
Al	0.019±0.010	–
S	0.022±0.035	1.123±0.077
Mg	–	0.862±0.012
Ca	0.054±0.180	0.158±0.122
Na	–	0.405±0.067
Si	0.128±0.441	0.039±0.650
Fe	0.022±0.381	0.035±0.431
Zn	0.015±0.061	0.034±0.078
K	0.021±0.081	–

References

Peng B Y, Su Y, Chen Z, Chen J, Zhou X, Benbow M E, Criddle C S, Wu W M, Zhang Y (2019). Biodegradation of polystyrene by dark (Tenebrio obscurus) and yellow (Tenebrio molitor) mealworms (Coleoptera: Tenebrionidae). Environmental Science & Technology, 53(9): 5256–5265

Yang L, Gao J, Liu Y, Zhuang G, Peng X, Wu W M, Zhuang X (2021). Biodegradation of expanded polystyrene and low-density polyethylene foams in larvae of Tenebrio molitor Linnaeus (Coleoptera: Tenebrionidae): Broad versus limited extent depolymerization and microbe-dependence versus independence. Chemosphere, 262: 127818

Zhang J, Kobert K, Flouri T, Stamatakis A (2014). PEAR: a fast and accurate Illumina paired-end read merger. Bioinformatics (Oxford, England), 30(5): 614–620